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TOXICITY OF DRILLING FLUID XP-07 ON MICROBIAL LOAD AND SURVIVAL OF *OREOCHROMIS NILOTICUS* FINGERLINGS

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ABSTRACT

Static bioassays were carried out in the laboratory for 96h to investigate the effect of Parateq a synthetic base mud on the fingerling of Orechromis niloticus and their microbial population. The fingerlings were exposed to different concentrations (0, 1000, 3000, 5000 and 9000Mg/l) of drilling fluid. Fish mortality data were recorded at 24, 48, 72 and 96h and analysed using Finney's Probit Analysis method to calculate LC_{50} values (concentration of drilling fluid in water that will kill 50% of the fish population in 96 hours). The 96h LC_{50} was 2210Mg/l.The mortality increased with increase in concentration, while, the lethal times decreased as concentration increased. The bacterial biomass ranged from 1.59 x 10^8 to 6.0 x 10^6CFU/ml while fungal biomass ranged from1.0 x 10^3 to 2.1 x 10^4 CFU/ml. Results revealed that there was an increase in fungal biomass with increase in mud concentration and a decrease in bacterial biomass with increase in mud concentration.

INTRODUCTION

In West Africa, Nigeria (Niger Delta) a lot of oil and gas exploration and development activities are carried out using drilling fluids. Drilling fluids are suspension of solid in liquid emulsion and/or dissolved materials with chemical additives which are employed during exploration to remove cuttings. Two types of fluids normally use in drilling operations are water based fluids and non-aqueous drilling fluids (OGP, 2003). Drilling fluid when discharged untreated into the environment are capable of interfering with the normal functions of the organisms (Saasen *et al.*, 2001; Wills, 2000). Drilling waste may kill marine life, smother or suffocate it with plume of suspended particles. This will result in potential damage to fish and invertebrate population and alteration in feeding and spawning grounds (Neff *et al.*, 2000; Soegianto *et al.*, 2008). Rhodes and Hendricks (1990) discovered that the components of drilling fluids inhibit the growth of microbial communities that are important in some biological cycles present in the ecosystem which may affect the productivity of such system.

In aquatic ecosystems, Fish are located at the top of the food chain and are highly visible resources. They are known to be affected by pollutants due to their being in direct contact with water via their gills and body surface. Fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and possible contaminants and alterations (Yousuf *et al.*, 2008). The estimation of bacterial numbers in fish is frequently used to retrospectively assess microbial quality or to assess the presumptive safety of the product (Okonko *et al.*, 2008). When microbial growth and actions are affected by the presence of contaminants in the aquatic system, the fishes are exposed to

harmful organisms which are subsequently passed on to man.

Ekpo and Uzegbu, (2004) studied the effect of drilling additives (chromelignsulphonate) on *Pachymelania fusca* and *Tympanotonus fuscatus* and observed an increase in the microbial biomass with increase in concentration of drilling additives. However, little information exists on effect of drilling fluid and it's additives on microbial load of fish. This study aims to determine any significant changes in the bacterial and fungal biomass in *Oreochromis niloticus* when exposed to Parateq a commonly used drilling fluid in the Niger Delta wetlands of Nigeria.

MATERIALS AND METHODS

Fingerlings (8.45cm) of *Oreochromis niloticus* were obtained from the African Regional Aquaculture Centre (ARAC), Aluu, Rivers State - Nigeria.

In the laboratory, the fish were transferred immediately to the holding tanks [120cm x 120cm x 120cm]. The holding tanks were aerated, cleaned and the water renewed daily (Reish and Oshida, 1986). Fish were fed twice daily with Nigeria Institution of Oceanography and Marine Research feed (NIOMR) (35% protein).

Drilling fluid was obtained from Bariods Nigeria Ltd and prepared in advance. The fluid was stored in a 5 litre bucket at approximately 5^0C. Immediately before the fluid was used, it was mixed in a storage bucket with a mixer at 3450 rpm for ½ hour to ensure sample homogeneity.

A complete randomised designed was used and the tests were conducted in the laboratory under room temperature using static non-renewal bioassay (Ca1/EPA, 2004). The solutions (0000Mg/l, 1000Mg/l, 3000Mg/l, 5000Mg/l and 9000Mg/l) were thoroughly mixed for 20 minutes and allowed to settle for 1 hour before the introduction of fish (Reish and Oshida, 1986, FAO, 1987).). The drilling fluid settled into a thin layer covering the bottom of the test chambers.

One hour after the preparation of the test solutions, ten healthy organisms were randomly selected and placed in the tanks in 3 replicates and covered with a net that was fastened with a rubber band to prevent fish escape. The fish were not fed during the 96hrs bioassay. Water quality parameters (Temperature, Alkalinity, pH and Dissolved Oxygen) of the test tanks were monitored daily for the replicates using standard methods (APHA, 1998).

Mortality was used as a measure of toxicity in terms of 96 hrs LC_{50}.The numbers of dead and active fishes were recorded. A fish is presumed dead and removed immediately when there are no opercula or body movement after gentle prodding.

At the end of the 96 hours, the total viable count (TVC) of heterotrophic bacterial and fungal population on the fish body was determined using nutrient agar (APHA, 1998). The average counts of colony forming unit (CFU /ml) were calculated and recorded.

RESULTS AND DISCUSSION

The various physicochemical parameters (Temperature - 28.15 ± 0.65; pH – 6.61 ± 0.31; Alkalinity – 23.38 ± 0.019; Dissolved Oxygen – 3.83 ± 0.28) did not vary significantly ($p > 0.05$) from those of the control except a slight decrease in the Dissolved oxygen (DO) which Ifeadi *et al* (1985) attributed to the effect of oil based fluid.

No mortality was observed in the control, however, in the other treatments, mortality increased with increase in drilling fluid concentration over time. At 24h, no death was recorded at the lowest concentration of Parateq. However, as the time of exposure increased, more deaths were recorded. At the end of 96h, the result showed 26, 33, 73 and 80% mortality recorded in 1000, 3000, 5000

and 9000 mg/l respectively (Fig. 1). The LT_{50} also decrease with increase in concentration.

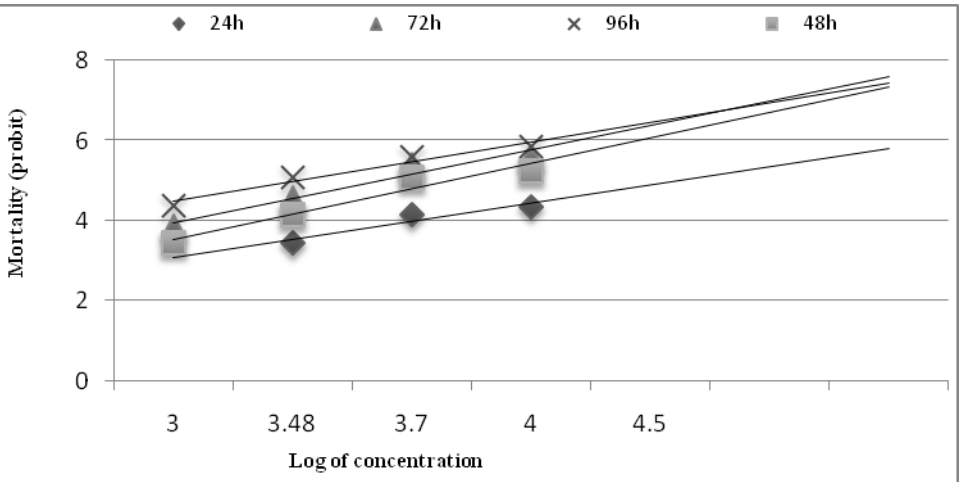

Fig. 1: Mortality in probit of *O. niloticus* exposed to different concentrations of Parteq drilling fluid 96 hours

The results indicated that as the concentration increased, more deaths were recorded and as the time increased the LC_{50} reduced (Table 1). The toxicity of Drilling fluids has been attributed to their hydrocarbon content (Neff *et al*, 2000; Tagatz *et al.,* 1985). Low Hydrocarbon content in the drilling fluid can be responsible for the low toxicity and also Synthetic based fluid does not readily disperse in water (Wills, 2000). Parrish and Ward (1988) recorded LC_{50} range of 0.45-39% using laboratory prepared drilling fluid on mysid and posited that the increased toxicity was due to the presences of hydrocarbon. The 96hrs LC_{50} value of the static bioassay with Parateq was 2210 mg/l (1% = 1000 mg/l), this LC_{50} was relatively low. This is probably because the test was not renewed daily during the 96h bioassay period (Neff *et al.,* 1981).

Table 2 shows the heterotrophic fungal and bacterial counts of *O. niloticus* exposed to Parateq. The total fungal counts ranged from 1.0 x 10^3 to 2.1 x 10^4 CFU/ml where the highest counts were found in fish exposed to 9000Mg/L. The fish in control tanks and in concentration 1000Mg/L had total viable count of 1.0 X 10^3. While in the other concentrations, the cells increased from the lowest to the highest concentration. The bacterial count decreased with increase in Parateq concentration. The fish in control had bacterial counts of 1.59 x 10^8CFU/ml while those in highest concentration (9000Mg/L) had a bacterial count of 6.0 x 10^6CFU/ml. The total counts for all the samples examined in this study were generally high exceeding the FAO/WHO Standard limits of 1.0 x 10 ^{2} CFU/ml that can be present in fish (ICMSF, 1986). This indicated that there is presence of harmful microorganisms on the fish body. Similar trend of bacterial growth was reported by Ekpo and Uzegbu, (2004) where the coliform counts ranged between 5.4 x10^2 and 8.5 x10^5 for shrimp and 5 x 10^2 to 4.4 x 10^4 cells for prawn when exposed to drilling mud additives. They further stated that when the total count reaches 10^6 CFU per gram or millilitre, the product is assumed to be, or nearing, spoilage. As noted by earlier workers

(Smith *et al*., 1992), micro organisms are usually the normal flora on the body of the fish. Their growth and multiplication are either encouraged or inhibited by toxicants. In this study, the growth of the bacterial population was inhibited while the growth of the fungal population was encouraged. Nweke and Okpokwasili (2003) observed that 1% drilling fluid waste elicited differential toxic effects on four bacterial isolates from the mangrove swamps in the Niger Delta. This they attributed to the natural ability of some bacteria to biodegrade the base oil. When bacterial growth and action are hindered by the presence of drilling fluid, fishes are exposed to harmful materials.

CONCLUSION

Despite the importance and sensitive nature of the Niger Delta, the area is replete with oil exploration and exploitation activities, which involve the use of drilling fluids. Most of the cuttings produced contain considerable proportion of drilling fluids which are dumped at the well site or deposited into the water. Drilling fluids elicited differential effects on the microbial population on the fish body. This has been attributed to the natural ability of some bacteria to biodegrade the base fluid. Thus micro organisms can increase or decrease in number depending on the effect of base fluid on the species.

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Table 1: Median Lethal concentrations (LC_{50}) of Parateq on *O. niloticus* in 96 hours

Time (hours)	LC_{50}(Mg/L)
48	3310
72	2710
96	2210

Table 2: Viable count of colonies from the fungal and bacterial isolates

Concentrations (Mg/L)	Fungal Counts(CFU /ml)	Bacterial Counts(CFU /ml)
0 000	$1.00 \times 10^3 \pm 141.4$	$1.59 \times 10^8 \pm 79.2$
1000	$1.50 \times 10^3 \pm 282.8$	$1.55 \times 10^8 \pm 8.50$
3000	$1.00 \times 10^3 \pm 282.8$	$1.12 \times 10^8 \pm 15.6$
5 000	$1.70 \times 10^3 \pm 282.8$	$2.70 \times 10^7 \pm 8.5$
9000	$2.10 \times 10^3 \pm 424.3$	$6.00 \times 10^6 \pm 1.4$